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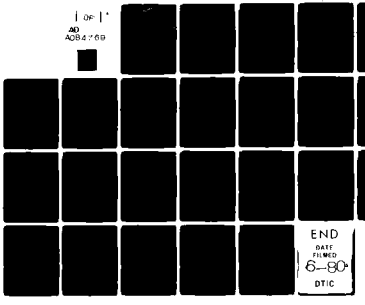

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(6) Characterization of the Chemical Constitution and Profile of Pharmacological Activity of PGB_x

by

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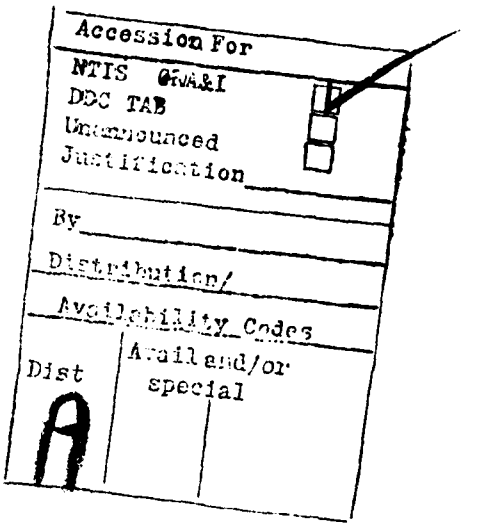


Studies on the Separation of the PGB_x Complex

This summary is to the date April 10, 1980 beginning after a late start in June 1979. The contract had a starting date of January 1, 1979 but inability to appoint the postdoctoral research associate delayed the study. Dr. Sha'aban F. El-Naggar was appointed to this position, and his extensive experience in the isolation of biologically active constituents from complex natural mixtures was valuable in testing a variety of separation methods that he was familiar with. The sample of PGB_x (acid form) that was used in this study was that prepared and supplied by Dr. H. W. Schmuckler of the Biochemistry Branch, ACSPD, Naval Air Development Center. The most active fraction had his designation PGB_x #28, fraction 2. Also, some samples were prepared by Dr. D. D. Miller from prostaglandin PGB_1. The following procedures were investigated.

A. Thin Layer Chromatography and Paper Chromatography

After testing the most common adsorbents such as silica gel G, alumina, polyamide, and kieselguhr together with many different solvent combinations, it became clear that the mixture was not going to be readily resolved. Also, the results pointed to PGB_x as a very complex mixture. Detection was by uv irradiation of the developed plates; with long wavelength and fluorescent indicator, the zones are dark on a fluorescing background in a generally streaked pattern. Addition of a complexing agent such as silver nitrate was of some help by dividing the mixture into three zones, each of which was still a complex mixture. Admixing kieselguhr to silica gel appeared to decrease the streaking and pointed to the possibility of a liquid-liquid partition system might be successful. Paper chromatographic studies were

next applied. The systems that gave appearance of a separation did not provide the clear separations sought and at best only major zones, such as fast moving and slow moving regions, could be reproducibly obtained. The systems were very sensitive to water concentration and temperature. One of the systems which appeared to give acceptable separation was later found to form several different solvent fronts during development. The result was not a separation as such but each front carried essentially the same material, and falsely suggested a separation. The paper chromatographic systems were therefore abandoned, at least till some new ideas are had.

The most successful tlc system developed was with silica gel G and kieselguhr in the ratio 1:1 as the adsorbent, and the lower phase of the solvent system prepared from chloroform--methanol--water (60:15:4). One of the goals in developing a useful tlc procedure was to be able to transfer to a column separation, as well as to have available a simple monitoring method for large scale separations. The following section reports the transfer of this system to a column separation.

B. Adsorption Chromatography

A transfer of the tlc system to a column separation resulted in the elution curve shown in figure 1. The effluent fractions were examined by tlc using the same solvent system and adsorbent with detection by irradiation with uv light. A drawing of the actual tlc plate is figure 2. Clearly, the column had produced a separation, although the components of the first and major peak are not cleanly resolved. Consequently, these fractions were bioassayed separately in the mitochondrial oxidative phos-

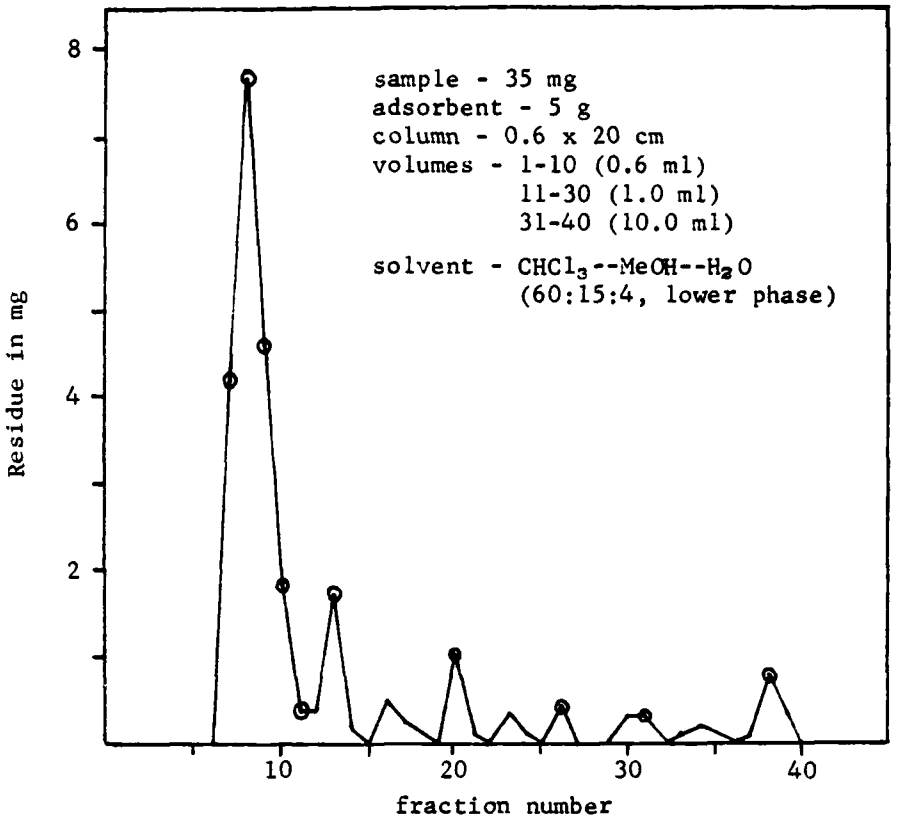


Figure 1. Chromatography of PGB_x on Silica Gel--Kieselguhr (1:1) Column

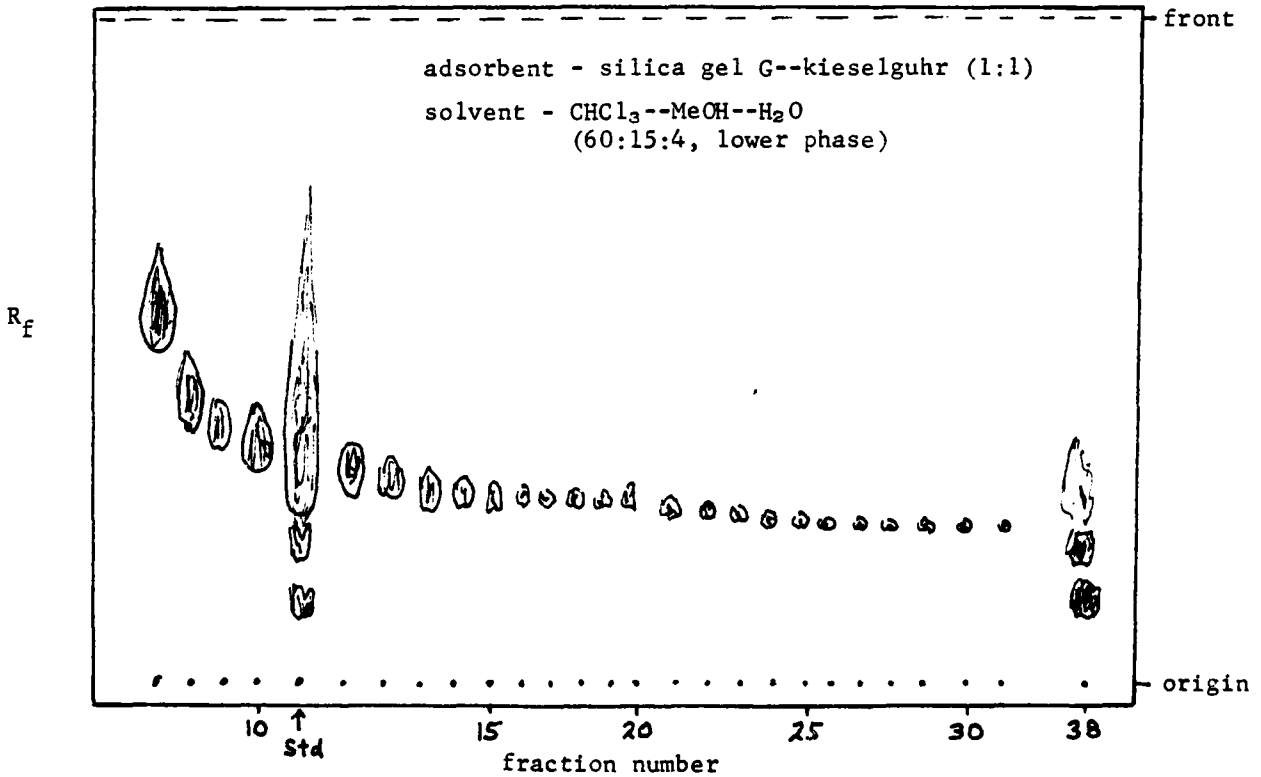


Figure 2. TLC Separation of Column Fractions

phorylation test. The results, as obtained from Dr. T. Devlin, are given in table 1.

Table 1. Bioassay Results of the Column Fractions from the Mitochondrial Oxydative Phosphorylation System

Column Fraction[a]	Percent Activity[b]		Comments
	20 μg	40 μg	
7	50	100	slightly lower specific activity
8	115	115	same as standard
9	105	125	slightly higher specific activity
10	120	125	" " " "
11	–[c]	130	" " " "
13	15	75	lower specific activity
20	–	135	higher " "
26	–	55	lower " "
31	–	100	same as standard
38	30	105	slightly lower specific activity

a The elution diagram (figure 1) has the fractions bioassayed indicated as circles.

b The percent activity is determined by comparing the test results with the results obtained from the crude PGB_X.

c Insufficient material available.

Although the bioassay results indicated some separation had occurred, the results were disappointing in that no fraction was completely inactive. We had expected to see at least one fraction that was inactive. The conclusion must be that the complex mixture of PGB_x is composed of similar substances with polarity differences that can be somewhat separated by the column system, and that the biological response is not particularly discriminating. There is, however, differences in specific activity for the individual components. At this stage, we decided to examine high pressure liquid chromatographic systems in the hope that a rapid and sensitive analytical procedure could be developed for the purpose of monitoring the macroseparations. In particular, a quantitative method would be very useful, since tlc is limited in this regard. A general summary of this work follows.

C. High Pressure Liquid Chromatography (HPLC)

The relative success of the tlc system indicated an adaptation of the same adsorbent and solvent might be a good first try for HPLC. An analytical column of silica gel (Corasil II of Waters Associates), a normal phase adsorbent, with dimensions 2 mm x 61 cm and 37-50 μ particle size was operated at 2000 psi and flow rate of 1 ml/min. The detector was set at 245 nm. The results showed one major peak with a retention time of 1.5 min, and considerable tailing. The solvent was $CHCl_3$--MeOH--H_2O (75:19:5). On decreasing the proportion of methanol, the new solvent composition of 75:15:5 increased the retention time to 2.5 min but no additional peaks or shoulders were observed. The tailing still was present. Elimination of water resulted in extensive streaking of the sample throughout the column; thus, water is absolutely necessary for any migration. A variety of other solvent combinations

and solvent changes were of little value in affecting the general profile of the elution curve. The use of the normal adsorption method did not appear to be a good procedure to pursue further.

HPLC with reverse phase columns was tried next. Of the variety of systems tried, the following was the best. A Waters Associates Bondpack C_{18} column (2 mm x 61 cm) with the solvent system of water--acetonitrile--chloroform--acetic acid (76.7:23:0.2:0.1) gave two peaks, one with retention time of 1 min, the other 4.3 min. The former was about 95% of the total sample applied, and the peaks were not symmetrical nor returned to the base line. The small peak was not seen when acetic acid was eliminated from the solvent system. Increasing the acetonitrile proportion at the expense of water to give the combination 55:44:0.9:0.1 resolved PGB_x into three fractions, but again streaking and unsymmetrical peak shapes were present. Modifying the solvent system did not improve the results significantly. Addition of a salt such as sodium acetate at various concentrations in the hope that some chelation might occur and thereby alter the migration was also tried, but no improvement occurred.

D. Partition Chromatography

After the disappointing results with HPLC, a review of the tlc experiments was made. Since the separation was improved when the proportion of silica gel was decreased and kieselguhr increased, it appeared that the relatively clear zones were possibly obtained by a liquid-liquid partition process rather than by adsorption. By employing the solvent system of chloroform--methanol--water (60:15:4) which as the lower phase (organic) was used in the tlc experiments, a partition column was prepared with kie-

selguhr as the binding matrix and the upper phase (aqueous) as the stationary phase. The lower phase was the mobile phase. The material that moved through the column was divided into three fractions. These have been sent for assay in the mitochondrial system. Results are not yet available.

In this connection, a novel technique known as drop countercurrent chromatography (DCC) may be of value. A demonstration of the procedure was seen (by R.W.D. and S.F.E.) in Aug. 1979 and a request for equipment was made to the university, but funds were not available. Subsequently, Dr. N. Werthessen had informed us about a successful separation of the inhibitor from the active fraction by this procedure. A request has been made to readjust our budget and to supplement our contract at the end of the year so that the equipment can be purchased immediately. (After this report was written, we were informed that approval was granted.)

E. Preparation of PGB_X and Separation on Sephadex LH-20

PGB_X was prepared from PGB_1 by Dr. D. Miller according to the established method and the product (290 mg) was separated with methanol on a column of Sephadex LH-20 (115 g). As the column effluent was collected, a monitoring of the fractions was performed by our tlc system. The results of the experiment are seen in figure 3. Clearly, the product is a complex mixture (as we already knew, but the picture conveys well the problem before us). Four major fractions (each of about 50 mg) were formed and submitted for testing in the mitochondrial system. The test results indicated all four were as active as the control. This showed that Dr. Miller was able to prepare the same PGB_X material as supplied to us by Dr. Schmuckler, but again we saw no real separation of the activity into different fractions.

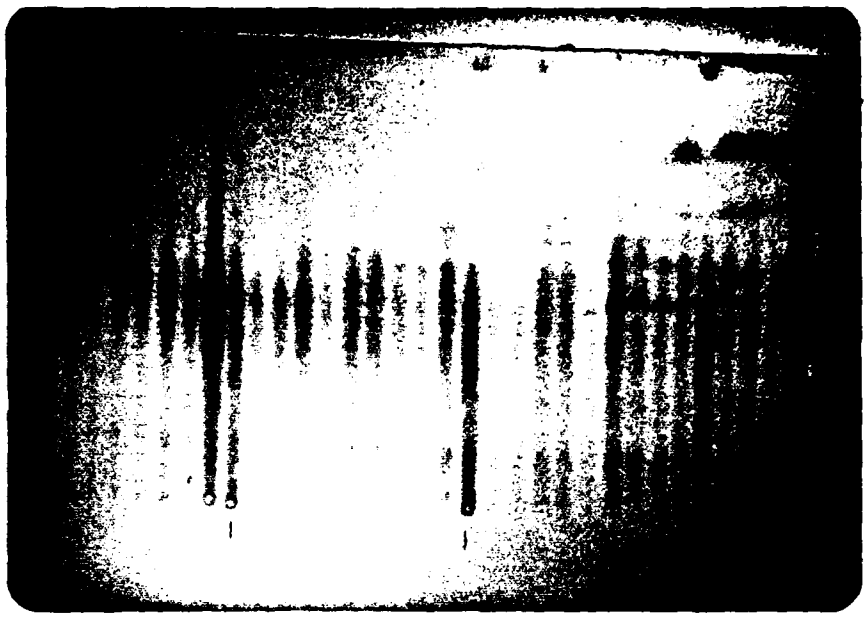
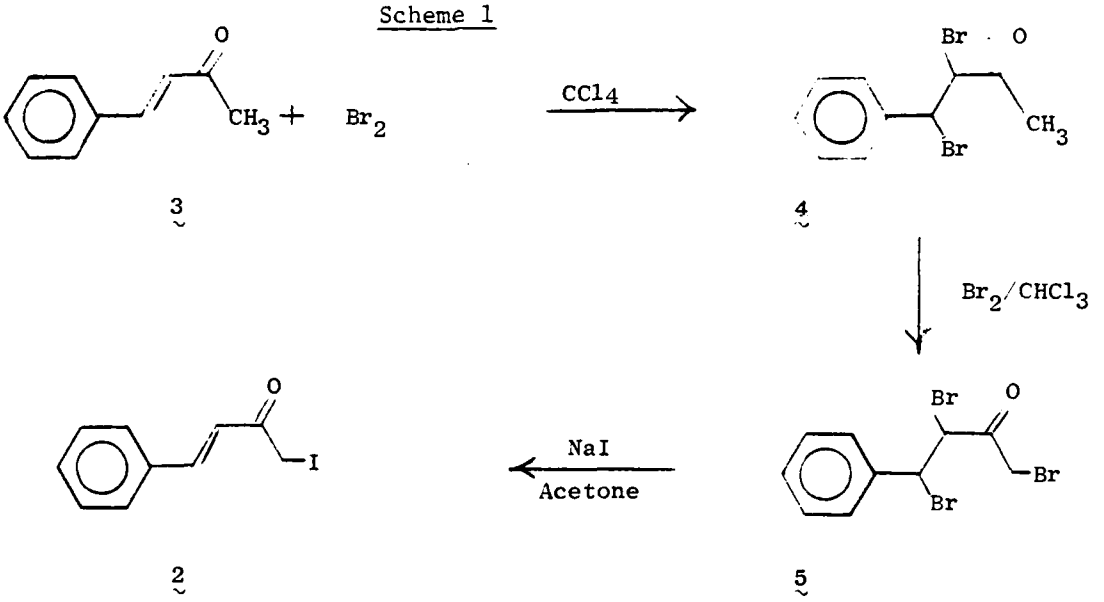


Figure 3. TLC Separation of Fractions from a Sephadex LH-20 Column (The longer vertical lines at base of TLC plate indicated at every tenth spot is the standard mixture before separation.)

Synthesis PGB_x

An important step in the synthesis of PGB_x involves the treatment of the methyl ester of 15-keto PGB_1 (1) with potassium hydroxide in aqueous ethanol (Polis et al., 1979). We became interested in the synthesis of the methyl ester of 15-keto PGB_1 due to the lack of an economical source of this material and due to the number of steps involved in the synthesis of 15-keto PGB_x (Polis et al., 1978) along with the fact that other procedures do not appear feasible on a large scale (Miyano, 1970). We utilized as one of our important starting materials, 1-iodo-4-phenyl-3-buten-2-one (2), which had previously been reported by Southwick et al., (1950) and its synthesis is outlined below:

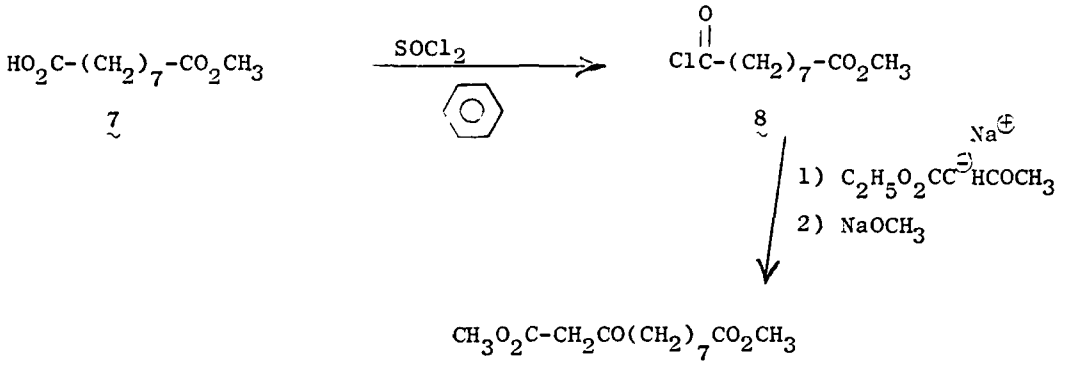
Scheme 1



In this known synthetic sequence, 4-phenyl-3-buten-2-one is treated with bromine in carbon tetrachloride and the resulting solid 4 is recrystallized from methanol. The dibromide 4 is allowed to react with a second molecule of bromine in chloroform to give the tribromide 5. Treatment of 5 with sodium iodide in acetone at room temperature for three hours gives the desired 1-iodo-4-phenyl-

3-buten-2-one 2. The second known compound to be utilized is dimethyl 3-oxoundecan-1,11-dioate 6 (Miyano; 1970) and the preparation of this material is illustrated in Scheme 2.

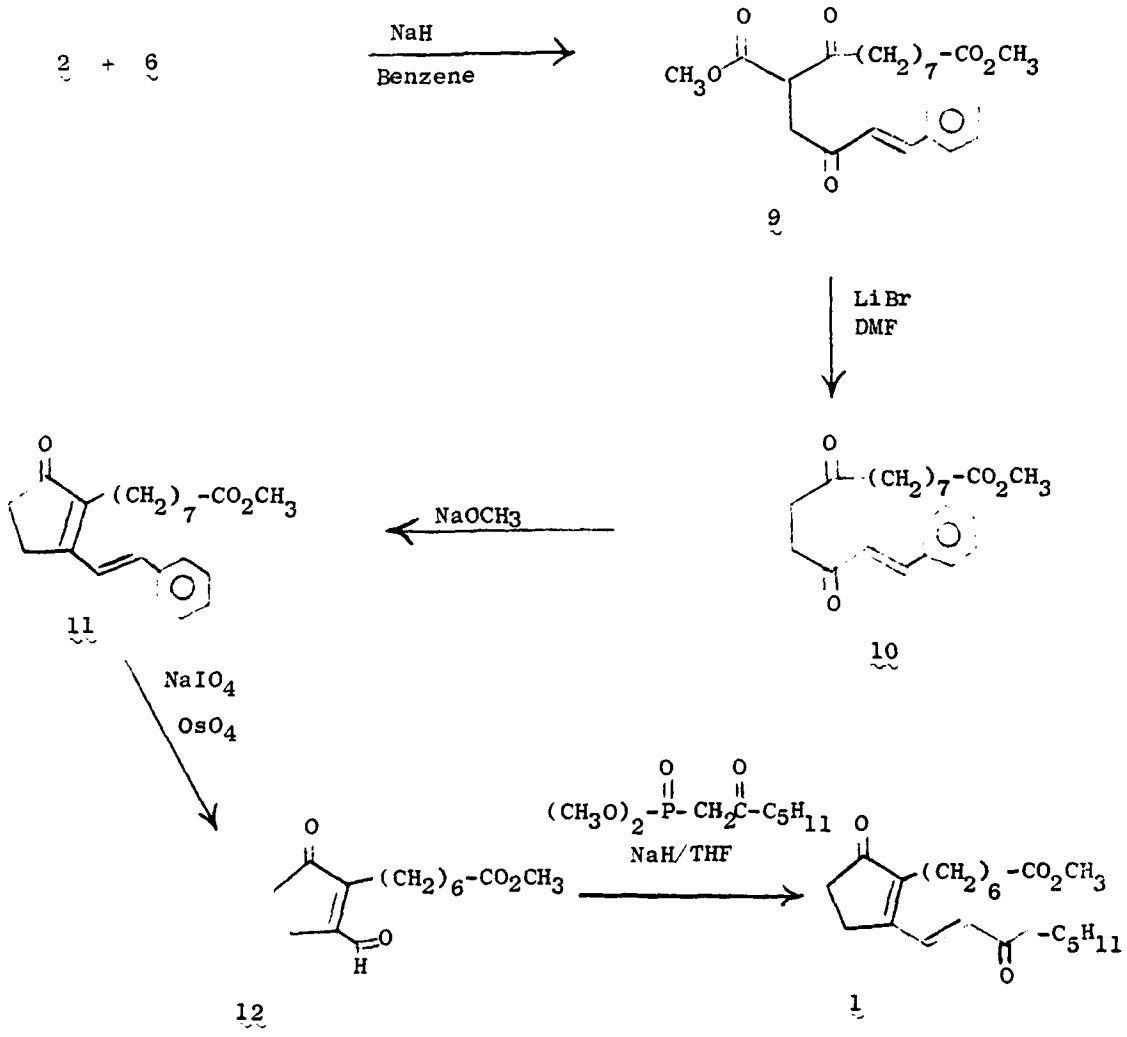
Scheme 2



Azelaic acid mono methyl ester 7 obtained commercially is converted to the acid chloride 8 by treatment of 7 with thionyl chloride in refluxing benzene for four hours. Pure 8 is obtained by distillation under reduced pressure. The acid chloride 8 is allowed to react with the sodium salt of ethyl acetoacetate followed by treatment with sodium methoxide in dry methanol to give crude 6. The diester 6 is purified by silica gel chromatography using a chloroform: ethyl acetate solvent system. The two known compounds 2 and 6 were then combined as illustrated in Scheme 3 by treatment of the diester 6 with sodium hydride in benzene followed by the addition of iodoketone 2 to give the desired ester 9 in an 90% yield. Initially, we tried a direct conversion of 9 to 11 but this attempted conversion failed. We then attempted a variety of methods to carry out the decarbalkylation of 9 to 10. Initially, we found that the

method of Krapcho et al., 1974 was a useful procedure but have recently found the treatment of 9 with lithium bromide or lithium iodide in dimethyl formamide provides a better yield of the desired product 10. At the present time, we are investigating methods for the cyclization of 10 to 11 under a variety of conditions. Thus far, we have not been satisfied with the use of sodium methoxide in methanol and we are examining alternative procedures. Once compound 11 has been isolated in large quantities we plan to convert it by osmium tetroxide-catalyzed periodate oxidation to give aldehyde 12 and treat this material with the commercially available Wittig reagent, dimethyl (2-oxoheptyl)phosphonate, to give the desired methyl ester of 15-keto-PGB_1.

Scheme 3



We have investigated the conversion of the methyl ester of 15-keto-PGB_1 to PGB_x using a variety of conditions. At the present time, we have found that treatment of methyl ester of 15-keto-PGB_1 in ethanol with KOH overnight at room temperature gives a product (OSU-KM-11) with a UV change from 296mn to 240mn. A product (OSU-KM-12) obtained by heating the methyl ester of 15-keto-PGB_1 in ethanol with KOH for four hours at 80°C provided a similar type UV change (296 to 241nm). However, we have noted a considerable difference in the thin layer chromatography pattern between the two products formed from using the different conditions. We have also found that the product (OSU-KM-12) formed from the overnight reaction at room temperature if placed in ethanol with KOH at 80°C for four hours gives material (OSU-KM-13) with a thin layer chromatography pattern very similar to the product isolated from a single treatment of the methyl ester of 15-keto-PGB_1 in ethanol with KOH for four hours. Products from each of these reactions were evaluated for their biological activity by Dr. T. M. Devlin at Hahnemann Medical College. (See Table 1).

TABLE 1

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Fraction \ ug/2.8 ml	activity* 20	40	80	Solution	Comments
OSU-KM-11	20	20	65	Slightly Hazy; Pale	Less activity than standard PGB_x; may contain inhibitor
OSU-KM-12	(-33)	45	95	Clear; deep honey color	As active at high concentrations; may contain inhibitor
OSU-KM-13	65	65	90	Clear; colored	Less active than control at lower concentrations

*Activity as percent of the effect of an equal quantity of PGB_x (lot #27) to stimulate phosphorylation of rat liver mitochondria.

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Studies on the Pharmacology of PGB_X

PGB_X was supplied by Dr. H. W. Shmukler, Biochemistry Branch, ACSPD, Naval Air Development Center,* as a dark amber alcoholic solution containing 155.6 mg of PGB_X acid per ml. It was converted to the water-soluble sodium salt by us and both the freeze dried salt and alcoholic solution were stored at 5°C in a dessicator. For all biological studies, PGB_X-Na was dissolved in appropriate volumes of Sorenson's phosphate buffer (0.0667 M), pH 7.4 or 7.6, and passed through a cellulose triacetate membrane filter (0.2 micron pore size) into sterile stoppered serum vials. All pharmacological studies were performed using PGB_X-Na although the doses and concentrations are expressed in terms of PGB_X acid.

Samples of PGB_XNa were submitted to Dr. T.M. Devlin, Hahnemann Medical College, for mitochondrial assay (1) and the results are portrayed in Table 1. Our sample is virtually indistinguishable from the reference standard used by Devlin and we are satisfied that we are working with authentic PGB_X.

As described in the original proposal, we have begun to examine the general pharmacological behavior of PGB_X in terms of a) acute toxicity, b) gross behavioral activity and neurological deficit, and c) some aspects of autonomic and cardiac function, the results of which are summarized in this report. We have also attempted to establish an organ system assay for PGB_X that will enable us to quantitatively evaluate the activity of PGB_X fractions and analogues.

*This sample was described by H.W.S. as Fraction 2 (Sephadex LH20 column separation) of preparation No. 28.

a) Acute toxicity. The lethal dose-response curves for PGB_x following intraperitoneal administration of single doses in male albino ICR mice [Hap:Swiss(ICR)BR, 18-22 gm] are graphically depicted in Fig. 1. The incidence of death is clearly time dependent during the first 96 hrs. A similar representation is depicted in Fig. 2 for the intravenous route of administration. The median lethal doses (LD50's) calculated from the curves are presented in Tables 2 and 3. The 96-hr LD50's are considered the best estimates of the acute lethal potency of PGB_x. The full lethal consequences of PGB_x administration are not seen until four days have elapsed (regardless of route of administration) and this suggests that a) the organ damage that causes death develops slowly, or b) the lethal determinant is a metabolite of PGB_x that can only accumulate over a period of time. It is not clear at present which of these possibilities is correct.

Fig. 3 illustrates the time-dependency of both IV and IP routes using another format. Animals are active, responsive and take nourishment during the 4-day postinjection period so that we believe they die from the "direct" effects of PGB_x (or its metabolites) and not as a consequence of depression-induced dehydration or starvation.

b) Gross behavioral activity and tests for neurological deficit in male albino ICR mice. Using the methods of Irwin (2) and Dunham and Miya (3), single intraperitoneal doses of PGB_x in excess of 25 mg/kg (equal to about one-fourth the LD50) produce a loss of alertness, spontaneity and reactivity. Some loss of abdominal tone is seen and movements tend to be uncoordinated and spastic during the first 24 hrs. Although the mice are fully conscious and active, their behavior is generally sluggish when

compared with control animals using a variety of behavioral testing paradigms. Doses less than 25 mg/kg produce virtually no acute abnormal behaviors.

c) Influence of PGB_x on cardiac contractility changes induced by inotropic drugs. We have examined the effects of PGB_x on the responsiveness of spontaneously-beating isolated right atria of both rats and guinea pigs, alone and in the presence of drugs that enhance the force of contraction (inotropy) of cardiac muscle. Our intent was two-fold: 1) to re-examine aspects of the cardiac response described by Apstein (4) that appeared to suggest that PGB_x accentuates the actions of the β-adrenergic agonist isoproterenol in isolated ischemic rabbit hearts during the post-ischemic recovery period, and 2) to develop an organ level biological assay that would serve as an alternative to the mitochondrial assay and that could more closely reflect activities that are therapeutically relevant.

Initial studies were performed on isolated rat right atrial preparations. Right atria were surgically removed from male Sprague Dawley derived albino rats of both sexes [Hap:(SD)BR, 200-500 gm] and suspended under 0.5 gm tension in a 10 ml tissue bath containing Kreb's solution aerated with 95% oxygen-5% carbon dioxide. Temperature was maintained at 36 ± 0.1°C. Rate (chronotropy) and force (inotropy) were monitored with either Grass or Narco Biosystems oscillographs equipped with isometric transducers.

Relatively high concentrations of PGB_x (e.g. 100 µg/ml bath) exert a mild increase in contractility (12 to 15% max), an effect which is not seen, or only occasionally seen, at lower concentrations. No rate changes were observed. When these tissues were preincubated, with varying

concentrations of PGB_x and subsequently challenged with a beta stimulant, such as (-)-isoproterenol, a striking increase in the force of contraction was observed. The largest increase in contractility occurred at the "top" of the dose-response curve (i.e. when isoproterenol concentration was high), suggesting that PGB_x increases the apparent intrinsic activity of isoproterenol. This activity was related to the concentration of PGB_x (8-12 µg/ml bath produced significant and what appeared to be maximum effects).

Histamine, a drug whose inotropic action is initiated by a mechanism different from isoproterenol, also produced an increased force of contraction that is intensified by PGB_x. (Histamine does not produce a positive inotropic action in rats so only guinea pigs were used for this study). Although there is some quantitative difference between the effects of PGB_x on isoproterenol and histamine, the results are qualitatively similar and strongly suggest that PGB_x operates by a mechanism that is independent of adrenergic or histaminergic membrane receptors. Some of our earliest guinea pig data portraying the effects of 9 µg of PGB_x/ml bath on both isoproterenol and histamine-induced contractions are presented in Fig. 4.

The contractility-enhancing actions of PGB_x provided the basis for an organ bioassay for the compound and additional studies were undertaken to establish assay validity and to determine the protocol necessary to maximize assay reliability.

Parameters examined were 1) influence of PGB_x incubation time, 2) dose-responsive range, 3) slope of the dose-response curve, 4) duration of PGB_x action and degree of recovery of tissue from PGB_x effect, and 5) magnitude of error associated with potency estimates.

Although it was possible to use the enhancement of either (-)-isoproterenol or histamine as the basis for the organ assay, we chose the isoproterenol system solely because we have a somewhat better understanding of its dynamics.

Fig. 5 illustrates the type of effect PGB_x exerts on guinea pig right atrial contractility in the presence of (-)-isoproterenol. Tissues were incubated with PGB_x (8 µg/ml bath) 3 min prior to the first addition of isoproterenol. The contractile agonist was introduced in graded increments until a maximum inotropic response is seen. This method of constructing cumulative dose-response curves was originally described by Van Rossum and van der Brink in 1963 (5). Note that the DR curves for isoproterenol generated before PGB_x treatment and 20 minutes after washing the tissue free of PGB_x are virtually identical.

Incubating the tissues with PGB_x for periods less than 3 or greater than 10 minutes (prior to isoproterenol addition) produce less intense responses. The dose-responsive range of PGB_x appears to be in the neighborhood of 2-12 µg/ml bath (Fig. 6). This is extremely narrow, and the bioassayist must subject all unknown compounds to preliminary dosing studies in order to estimate the concentrations that will fall within a dose-responsive range. On the other hand, the steepness of the dose-response curve makes it easier to discriminate between compounds having different potencies. The slope of the curve depicted in Fig. 6 is 38.13 ± 5.06 percentage units/log dose unit and the correlation coefficient is 0.758.

The bioassay protocol that has been developed appears to be reasonably reliable. Since PGB_x effect is rapidly terminated following

replacement of the bathing solution and since the atrial tissue recovers completely from the effects of PGB_x after equilibration with fresh medium for approximately 20 minutes, it is possible to use the same heart for repeated PGB_x trials. The one cautionary note that must be included here is that some hearts are inhibited by PGB_x and these should not be used in the assay. This selective exclusion process clearly prevents us from extrapolating our potency assessments to the heart population generally. However, it does allow us to make a valid relative potency determination for those PGB_x samples that exhibit some degree of positive inotropic behavior. In other words, the exclusion process does not diminish the assay's validity.

A latin square design requiring 2 concentrations of reference standard PGB_x and 2 concentrations of the compound of unknown potency (all falling within the dose-responsive range) has been adopted. Each heart is exposed to all 4 doses of PGB_x (each of which is challenged by 2×10^{-8}M of (-)-isoproterenol). The minimum amount of data necessary to make a potency evaluation requires 4 hearts (Table 4). A more reliable estimate of potency can be made by expanding the protocol to include 12 hearts (3 per dosing sequence). Obviously, limitations in the amount of compound available for evaluation will influence the type of protocol that can be used.

d) Effect of PGB_x on the vasopressor response to segmental stimulation of sympathetic outflow in adrenalectomized, pithed rats. In the course of a multidimensional activity screen, designed to examine the effects of PGB_x on systems responsive to known prostaglandins, we observed that PGB_x produced a significant inhibition of vascular muscle tone induced by electrical stimulation of innervating sympathetic nerves. Sixty male

Wistar rats [Hap:(WI)BR, 210-365 gm] prepared according to the method of Gillespie and Muir (6) were used. Rats which had been atropinized were anesthetized with ether, pithed and artificially ventilated. A stimulating electrode was introduced into the vertebral canal via the right orbit and an indifferent subcutaneous electrode attached through the dorsal skin surface. The stimulated region was at the level of T_9-L_i. Tubocurarine, IV, was administered and submaximal 1 msec, 20 volt pulses were delivered at graded frequencies for 14 sec periods at 2 min intervals. Systolic blood pressure was directly measured from the femoral artery (Statham P 23 10 transducer) and drugs were administered through a catheter inserted into the femoral vein. Both adrenalectomized and non-adrenalectomized rats were used.

The stimulus frequency-vasopressor response profile in adrenal ablated rats differs markedly from that seen in nonablated animals due to the massive release of catecholamine from the adrenals of the latter group (Fig. 7). In these acute experiments, PGB_x, PGB_1 and PGE_1 were examined. Fig. 8 depicts the effects of PGB_x on the frequency-vasopressor response at various time periods following drug administration. The data are corrected for changes in the control that are associated with deterioration of the preparation with time. Figs. 9 and 10 portray similar curves for PGB_1 and PGE_1, respectively. Although the experiments are limited to single doses of each compound, differences in the responses to these drugs can be noted.

In several chronic experiments PGB_x (1.2 and 6 mg/kg) was injected SC twice a day for seven days in rats that were subsequently prepared according to the method of Gillespie and Muir (6). Control rats received phosphate buffer vehicle in place of PGB_x. Frequency-pressor response

curves were generated for adrenal ablated animals (Fig. 11).

The influence of PGB_x and PGE_1 on heart rate and blood pressure in conscious rats was also evaluated using the method of Weeks and Jones (7). A polyethylene catheter was implanted in the abdominal aorta and exteriorized 6-24 hours prior to the experiment. Blood pressure and heart rate were monitored through this catheter. Intravenous injections were made through a catheter inserted into the left external jugular vein. PGB_x (1, 3 and 10 mg/kg) and PGE_1 (0.01, 0.03, 0.1 and 0.3 mg/kg) were injected at 10 or 20 min intervals (Figs. 12 and 13).

PGB_x, in single bolus IV injections of 10 mg/kg, inhibited the stimulus frequency-pressor response to electrical stimulation of sympathetic outflow from the spinal cord in adrenal ablated and nonablated rats. The inhibition exerted by PGB_x was slow in onset and progressed with time. Following SC injection of PGB_x for seven days, the frequency-pressor response to electrical stimulation decreased markedly and in a dose-dependent fashion. Doses of 1-10 mg/kg IV had no statistically significant effect on the basal heart rate and the blood pressure. PGE_1 produced a relatively short-lasting inhibition of the frequency-pressor response to electrical stimulation, and produces tachycardia and hypotension in conscious rats. On the other hand, PGB_1 (10 mg/kg IV) caused a significant augmentation of the pressor response to electrical stimulation in both adrenalectomized and pithed rats.

Thus, PGB_x, unlike PGE_1 and PGB_1, can persistently inhibit the sympathetic outflow of rats without significantly altering the heart rate and the blood pressure of conscious rats. We suggest that the decreased pressor response to electrical stimulation by PGB_x may be due to its

inhibitory action on the transmitter release from the sympathetic nerve terminals and what we are seeing may be related to the observation by Moss (8) that the "shock lung" syndrome provoked by cerebral hypoxia can be prevented by lung denervation or PGB_x. We are not yet in a position to identify the specific site or mechanism of this inhibitory action.

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Table 1. Mitochondrial oxidative phosphorylation assay of PGB_x sample (1AMB) and Devlin's reference standard (RS).

	PGB_x-Na-RS 38 µg[a]	PGB_x-Na-1AMB 38 µg[a]
ΔPi (µM)	17.6	14.7
	20	21
	17	16
$\bar{X}$ ± SE	18.2 ± 0.9	17.2 ± 1.9
%	100	94.7

[a]Expressed as the PGB_x acid.

Table 2. Acute lethality of PGB_x-Na, *IP*, in male albino ICR mice.

Time (hrs)	LD50 ± SE (mg/kg)[a]	N[b]
24	400 ± 41	39
48	170 ± 30	51
72	140 ± 30	51
96	90 ± 27	51

[a]Expressed as the PGB_x acid.

[b]Number of mice contributing to the curve from which the corresponding LD50 was interpolated.

Table 3. Acute lethality of PGB_x-Na, *IP*, in male albino ICR mice.

Time (hrs)	LD50 ± SE (mg/kg)[a]	N[b]
24	180 ± 26	29
48	91 ± 8	41
72	82 ± 6	35
96	69 ± 6	35

[a] Expressed as the PGB_x acid.

[b] Number of mice contributing to the curve from which the corresponding LD50 was interpolated.

Table 4. Dosing protocol for the organ bioassay of PGB_x.

		Dosing sequence			
		1	2	3	4
HEART	A	1[a]	2	3	4
	B	2	3	4	1
	C	3	4	1	2
	D	4	1	2	3

[a] 1 and 3 are low and high doses of the reference standard; 2 and 4 are low and high doses of the unknown preparation.

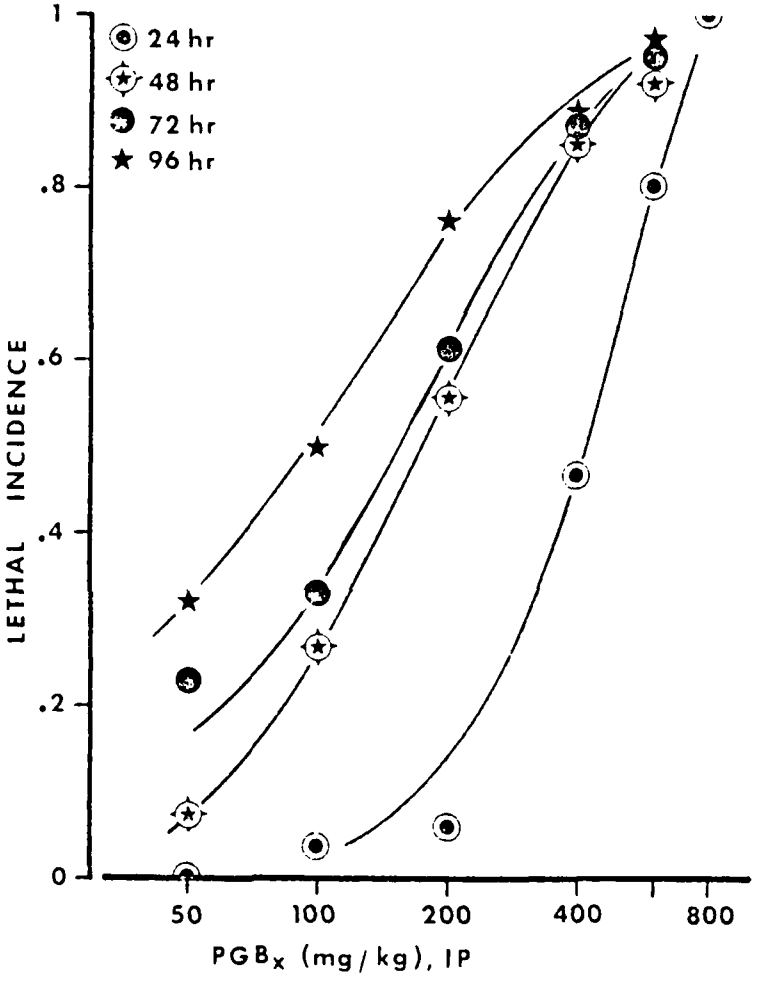


Fig. 1 Log dose response curves for PGB_X, administered IP, in male albino ICR mice. Doses are expressed in terms of PGB_X acid. Incidence of lethality (1 = 100%) varies with time of observation and maximum cumulative effect is seen at 96 hours. N = 39-51 mice/curve.

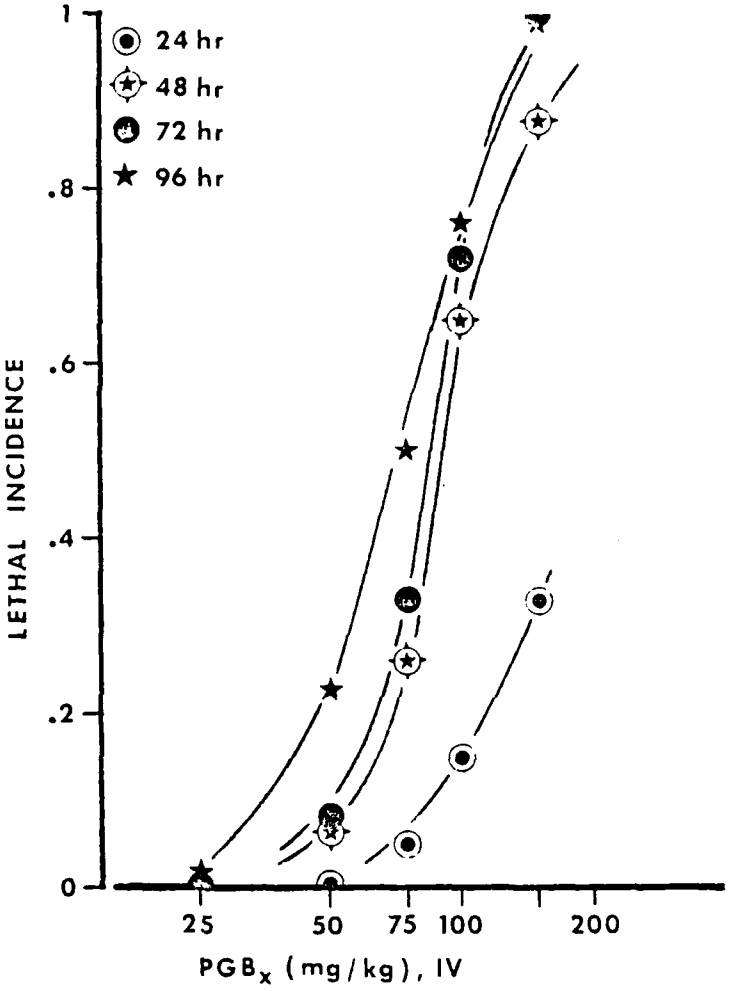


Fig. 2 Log dose response curves for PGB_x administered IV, in male albino ICR mice. Doses are expressed in terms of PGB_x acid. Incidence of lethality (1 = 100%) varies with time of observation and maximum cumulative effect is seen at 96 hours. N = 29-41 mice/curve.

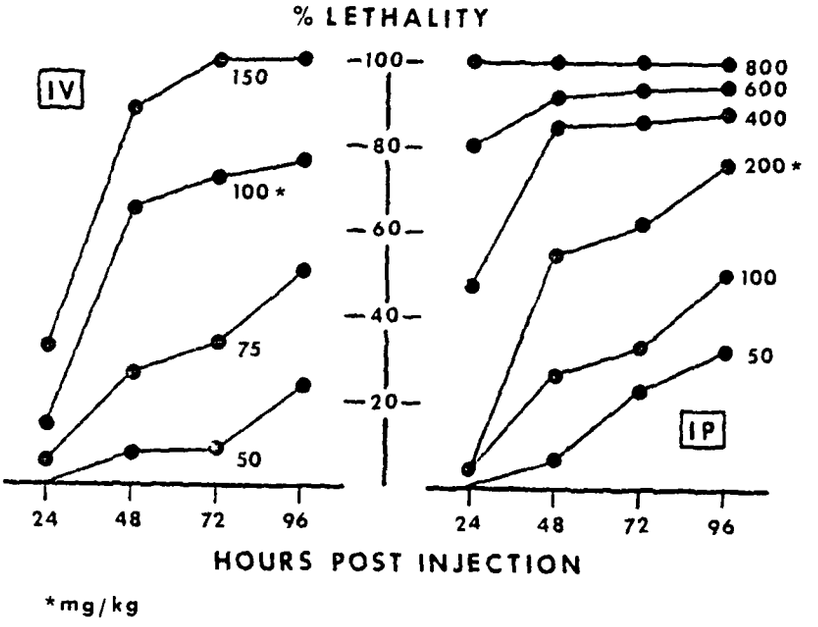
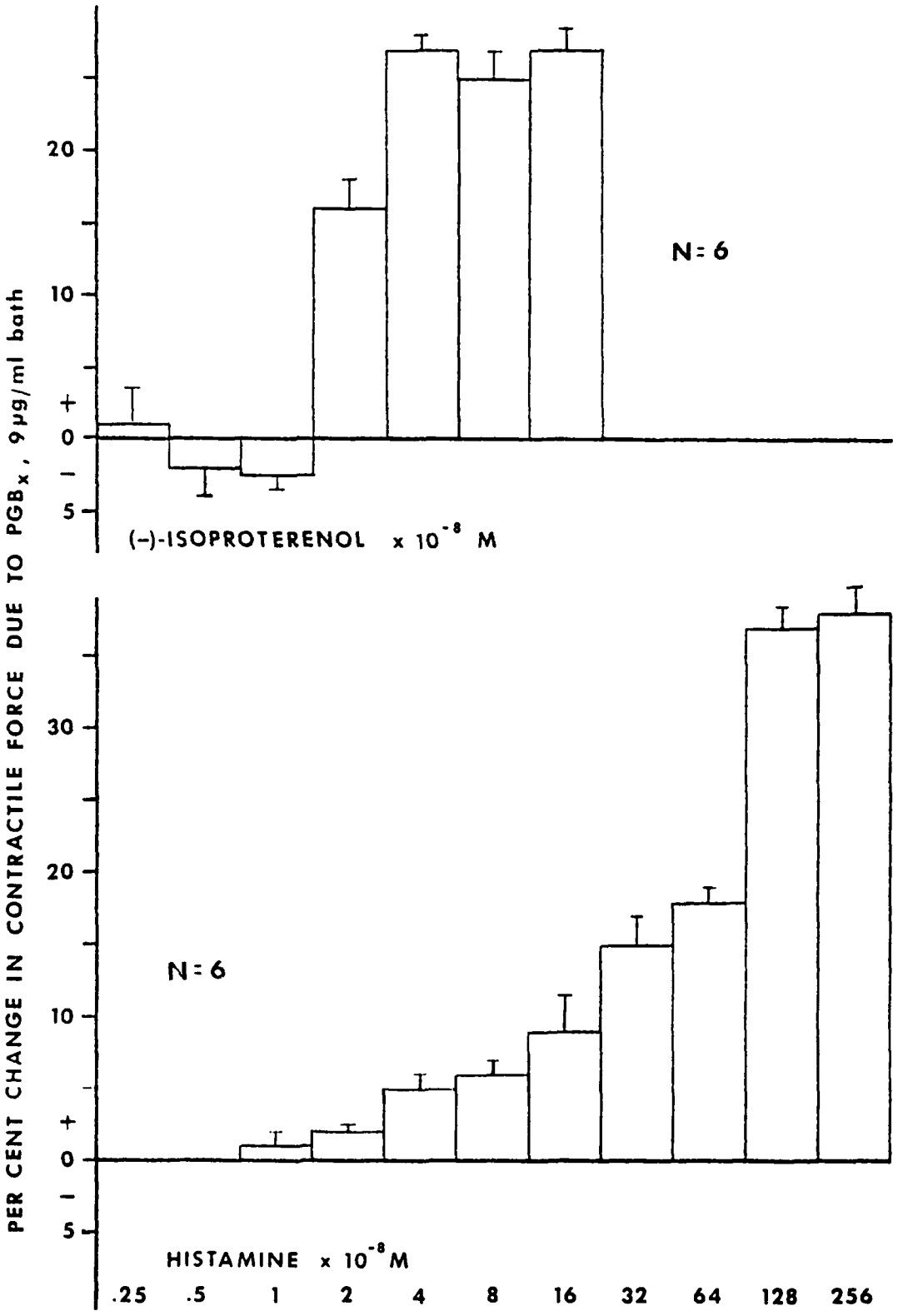


Fig. 3 Time-effect curves for PGB_x-induced lethality in male albino ICR mice. Incidence of death is maximum for all doses at 96 hours. N = 29-51 mice/curve.

Fig. 4 The influence of PGB_x on the response of the guinea pig right atrium to (-)-isoproterenol and histamine. Isolated right atria were incubated for 3 minutes with PGB_x, 9 µg/ml bath, and then exposed to graded doses of either isoproterenol or histamine. The same tissues had been previously incubated with phosphate buffer (control) and subsequently exposed to graded doses of isoproterenol or histamine. The differences between control and PGB_x responses are expressed as % changes in contractility attributable to PGB_x.

Fig. 4


PER CENT CHANGE IN CONTRACTILE FORCE DUE TO PGB$_x$, 9µg/ml bath
20
10
+
0
−
5
N = 6
(−)-ISOPROTERENOL x 10^{-8} M
30
20
10
+
0
−
5
N = 6
HISTAMINE x 10^{-8} M
.25
.5
1
2
4
8
16
32
64
128
256

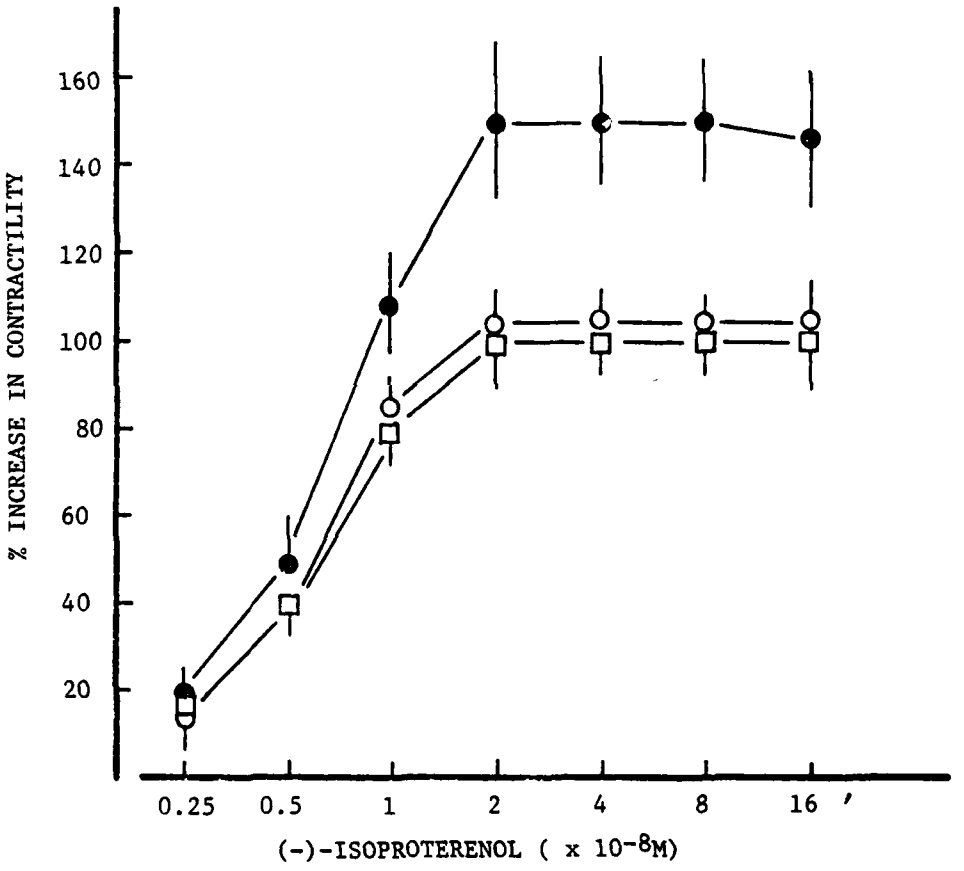


Fig. 5 Log dose-response curves for (-)-isoproterenol in the presence and absence of PGB_x. Spontaneously beating guinea pig right atria were incubated for 3 min with PGB_x before induction of the agonist. □ Agonist alone in the absence of PGB_x; ● PGB_x, 8 µg/ml bath; ○ agonist alone, 20 min after PGB_x washout (N = 6).

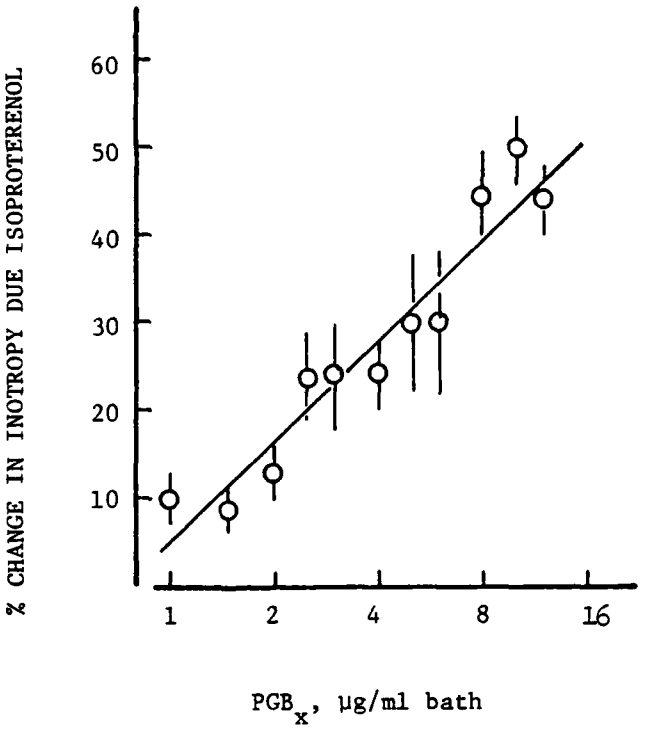


Fig. 6 Log dose-response curve for PGB_x on inotropic response guinea pig right atria to (-)-isoproterenol (2×10^{-8}M). Four hearts contributed to each datum point. Slope is 38.13 ± 5.06 percentage units/log dose unit; r = 0.758.

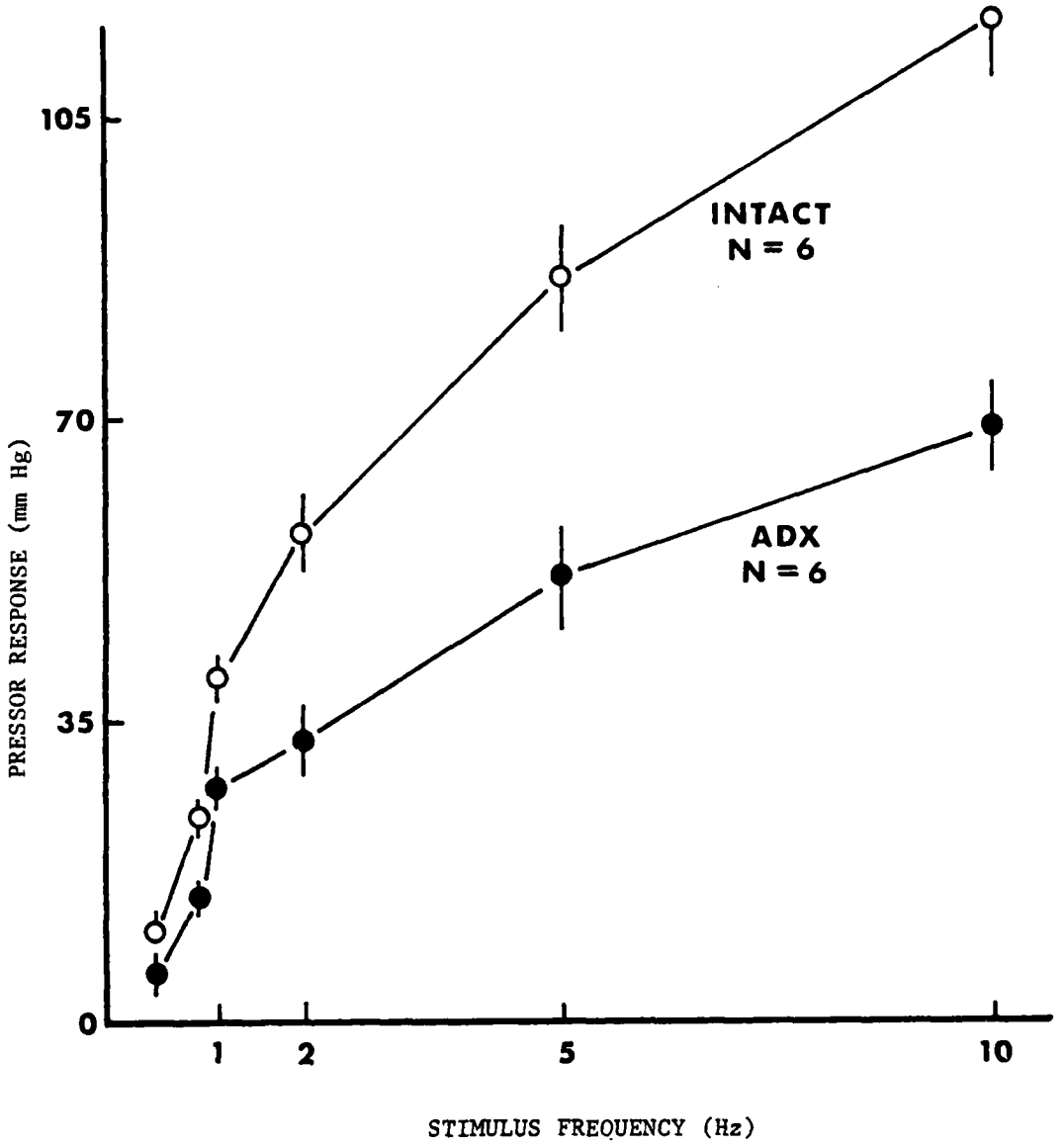


Fig. 7 Relationship between pressor response (change in systolic blood pressure from prestimulus baseline) and frequency of electrical stimulation of sympathetic vasomotor fibers in adrenalectromized, pithed rats (ADX) and non-adrenalectomized, pithed rats (INTACT).

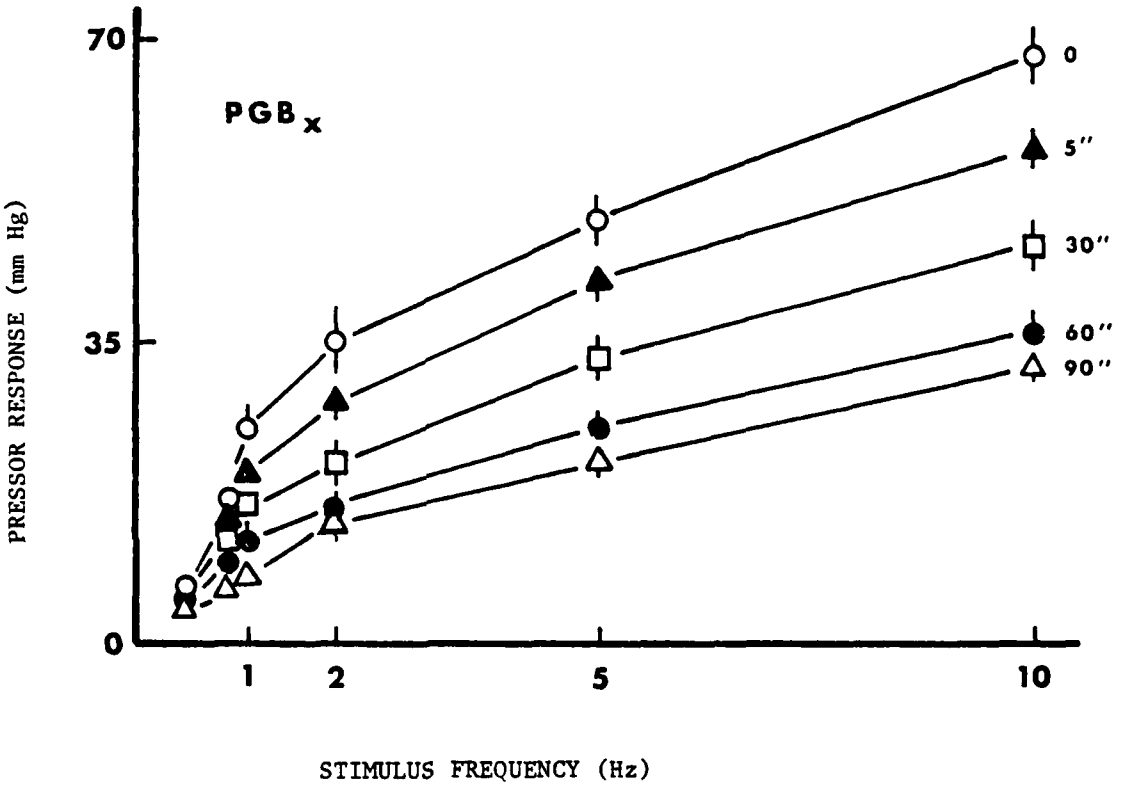


Fig. 8 Influence of PGB_x (10 mg/kg, IV bolus) on pressor response to electrical stimulation of sympathetic vasomotor fibers in adrenalectomized, pithed rats. Each curve represents a stimulus-response profile at a designated time after injection (N = 6).

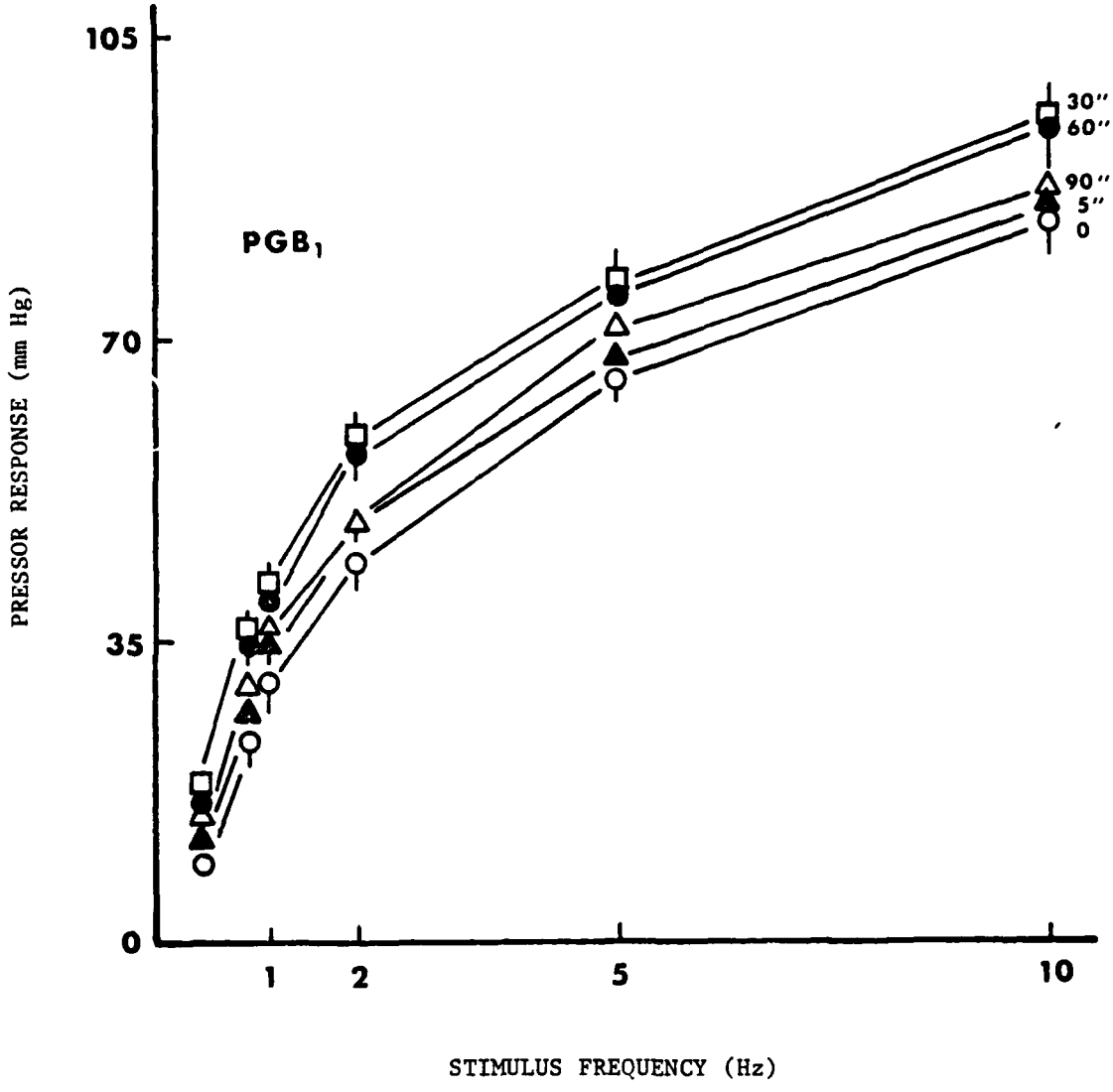


Fig. 9 Influence of PGB_1 (10 mg/kg, IV bolus) on pressor response to electrical stimulation of sympathetic vasomotor fibers in adrenalectomized, pithed rats. Each curve represents a stimulus-response profile at a designated time after injection (N = 5).

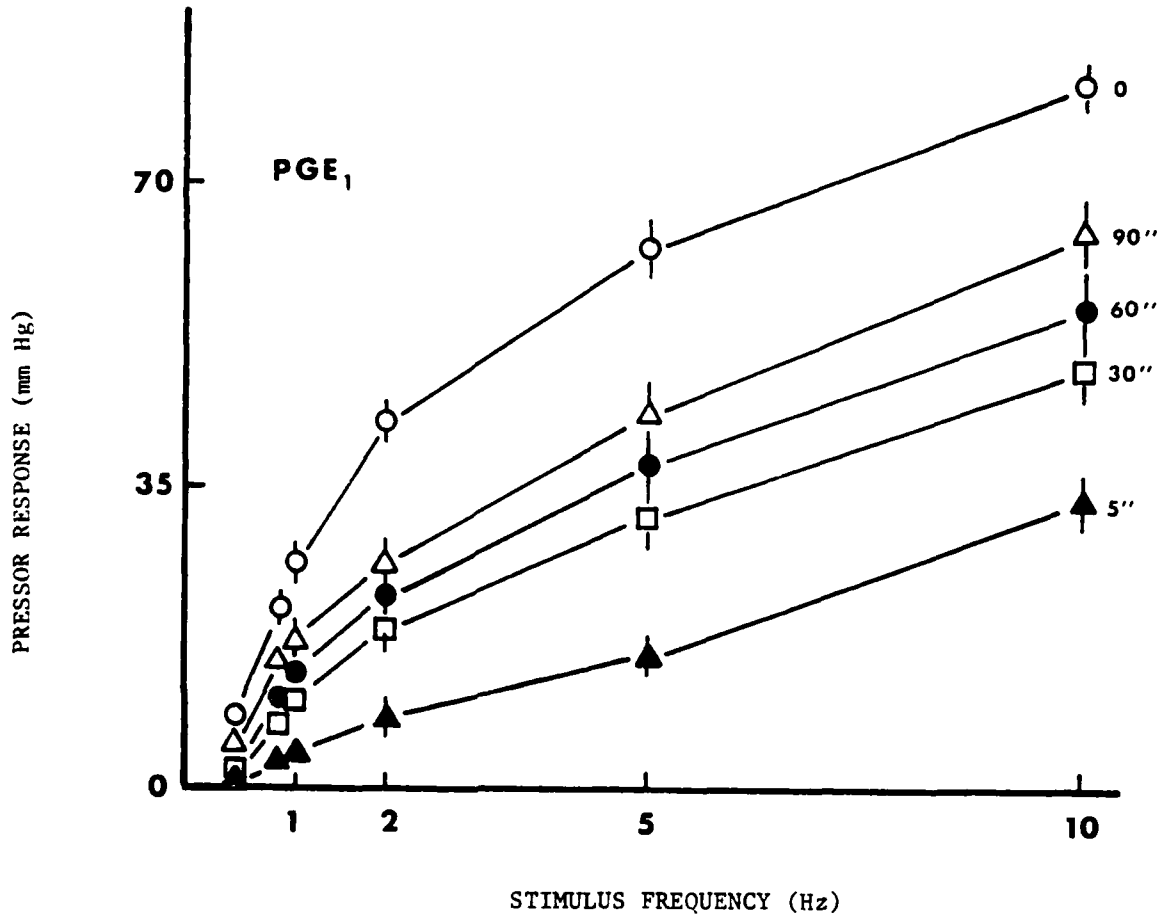


Fig. 10 Influence of PGE_1 (1 mg/kg, IV bolus) on pressor response to electrical stimulation of sympathetic vasomotor fibers in adrenalectomized, pithed rats. Each curve represents a stimulus-response profile at a designated time after injection (N = 5).

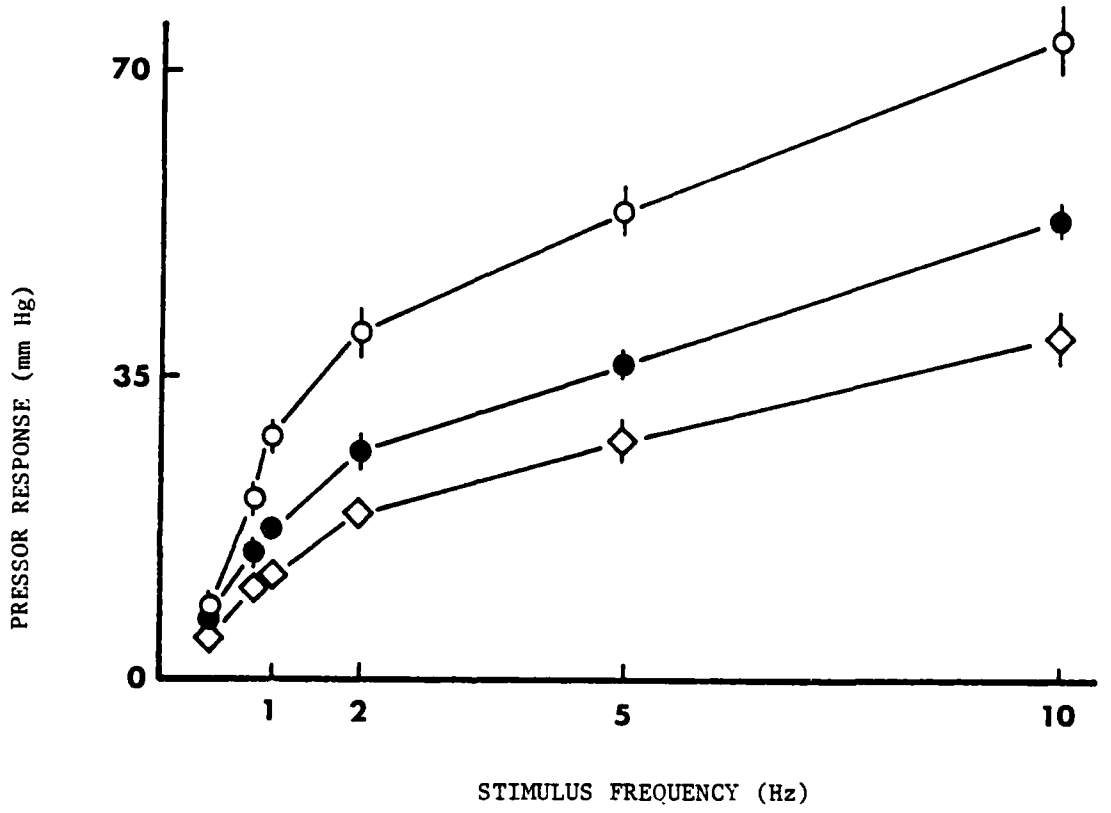


Fig. 11 Influence of chronic PGB_x pretreatment on pressor response to electrical stimulation of sympathetic vasomotor fibers in adrenalectomized, pithed rats. ○ Controls (N = 7); ● PGB_x, 2 x 1.2 mg/kg/day for 7 days (N = 5); ◇ PGB_x, 2 x 6 mg/kg/day for 7 days (N = 8).

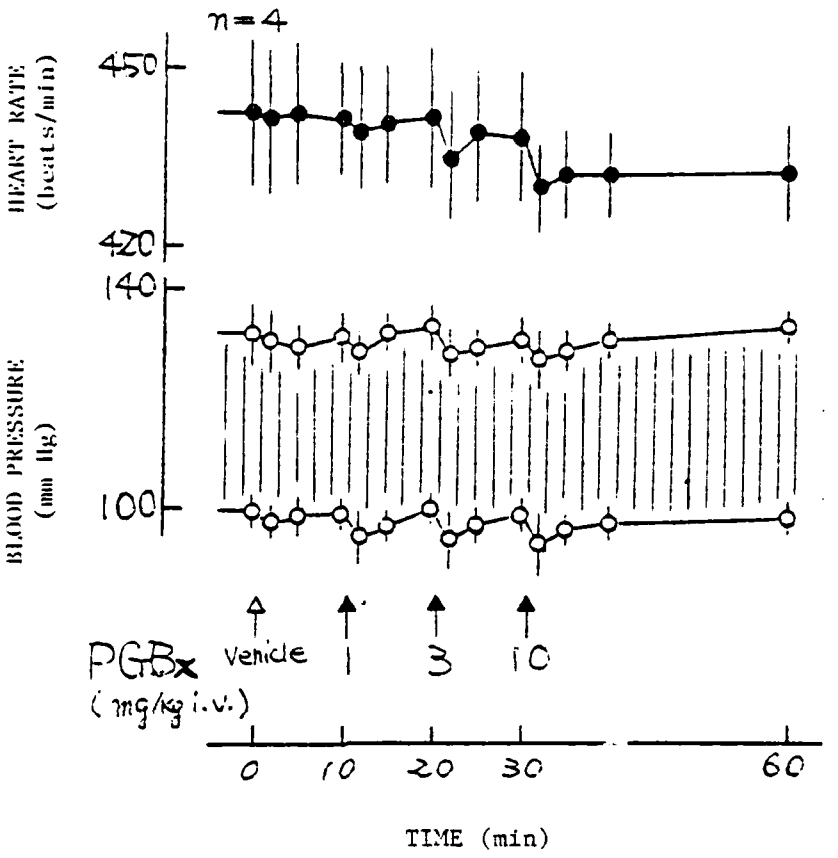


Fig. 12 Effects of bolus injections of PGB_x on heart rate and systolic and diastolic blood pressure in conscious, unrestrained rats.

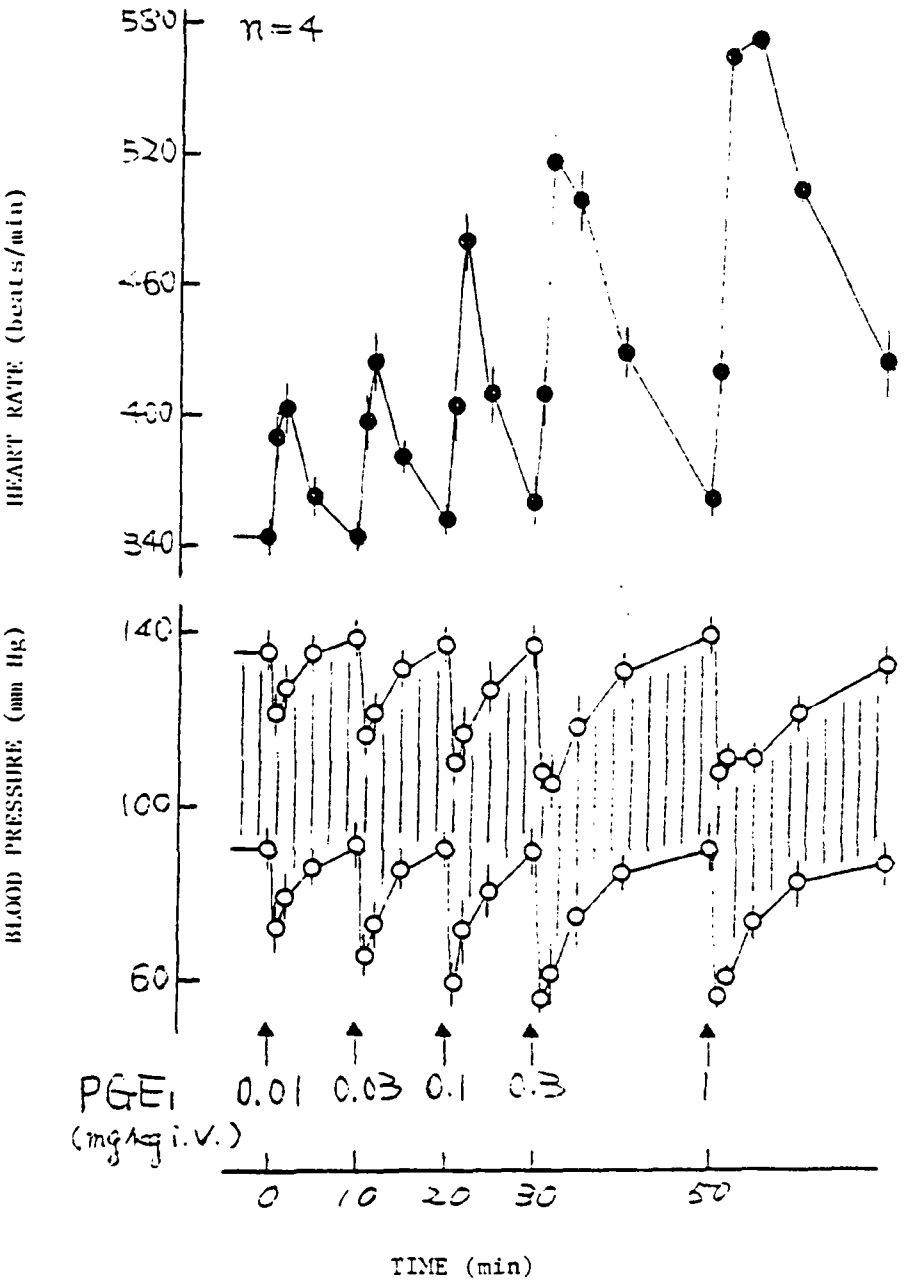


Fig. 13 Effects of bolus injections of PGE_1 on heart rate and systolic and diastolic blood pressure in conscious, unrestrained rats.

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prostaglandins, PGB_1, PGE_1, PGB_X, chromatography (thin layer, paper, column, high performance liquid), synthesis PGB_X, 15-keto-PGB_1 methyl ester, ultraviolet change, dimethyl 3-oxoundecan-1, 11-dioate, oxidative phosphorylation, spinal cord stimulation, inotropy, blood pressure, vasomotor tone.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

PGB_X, a synthetic polymeric derivative prostaglandin PGB_1, was examined by various chromatographic techniques, including thin layer, paper and high performance liquid chromatography. Fractions obtained from the separation methods were tested in the bioassay based on restoration of oxidative phosphorylation in aged mitochondria. Some separation of activity was observed but no fractions were devoid of it. The PGB_X mixture is highly complex containing very similar components based on polarity characteristics. Studies

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were made to shorten the synthetic sequence to PGB_x, with concentration on the synthesis of 15-keto-PGB_1 from benzalacetone. Seven of the eleven steps have been completed. Pharmacological testing of PGB_x was evaluated as affecting vasomotor tone and heart rate in: (a) conscious unrestrained rats, (b) adrenalectomized (ADX) pithed rats with exogenous norepinephrine, and (c) ADX and pithed rats subjected to segmental cord stimulation. PGB_x had no significant effect on B.P. or heart rate in a). PGB_x reduced the pressor response to electrical excitation of the cord in a time-dependent manner, and inhibited norepinephrine-induced pressor effects, but to a lesser degree. PGB_1 and PGE_1 produced short-lived augmentation and inhibition, respectively, of the pressor response. PGB_x inhibition is believed not to be acting by the blockage of adrenergic receptors but by interference with sympathetic nerve functions.

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